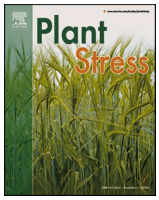
Plant Stress 16 (2025) 100803

Contents lists available at ScienceDirect

Plant Stress

journal homepage: www.sciencedirect.com/journal/plant-stress



Volatile organic compounds as potential markers of *Botrytis cinerea* infection in intact harvested grape berries

Pietro Emilio Nepi [a,§], Claudia Pisuttu [b,§], Cristina Nali [b], Elisa Pellegrini [b], Ron Shmuleviz [c], Stefano Brizzolara [a,*], Pietro Tonutti [a]

[a] Institute of Crop Science, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, Pisa 56127, Italy
[b] Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, Pisa 56124, Italy
[c] Department of Biotechnology, University of Verona, Strada Le Grazie 15, Verona 37134, Italy

ARTICLE INFO

Keywords:
Grey mold
VOCs
Wine grape
Postharvest withering

ABSTRACT

Partially dehydrated grapes are traditionally added in several wine-producing countries to enrich must composition for complex dry/sweet wines. Unfortunately, the controlled conditions in grape dehydration chambers are conducive to the development of *Botrytis cinerea* (causal agent of grey mold), thus resulting in significant grape losses. A few published papers have reported specific quantitative and qualitative alterations in the profile of volatile organic compounds (VOCs) of grape berries in response to *B. cinerea* infection. However, to the best of our knowledge, none of them studied the biochemical response of intact grape berries to the infection. The information deriving from intact berries analysis can be used to develop specific VOC sensors for early infection detection. To better understand the VOCs specifically induced by *B. cinerea* infection, homogeneous intact berries of non-inoculated Sangiovese and Corvina cultivars were collected and analysed by SPME-GC-MS. The same analysis was used for berries that had been artificially inoculated with a spore suspension of *B. cinerea* (105 spores mL^{-1}) or mock inoculated using the same volume of growth medium. The results showed that inoculated berries emit significantly higher levels of a set of primary (hexanol, 2-hexen-1-ol, 3-hexen-1-ol) and secondary (1-penten-3-ol) alcohols. Some of these alcohols have already been reported to correlate with *B. cinerea* infection, while others possibly representing new infection markers. Setting up sensors that can detect the volatile markers identified inside the dehydration chambers would improve grape withering through the early detection of *B. cinerea*, possibly leading to a reduction in spoilage and grape losses via the targeted adjustments of environmental conditions.

1. Introduction

Botrytis cinerea (Persoon: Fries) is a saprophytic and parasitic fungus, which is considered as the second most relevant plant pathogen worldwide for its scientific and economic importance (Scholthof et al., 2011). *B. cinerea* infections are very difficult to prevent, partly due to its different attack strategies and its ability to survive under different forms (i.e. mycelia, conidia or sclerotia) in crop residues (Holz et al., 2003).

Grapevine is one of crops that is most severely affected by this pathogen. *Botrytis cinerea* is responsible for leaf (Williamson et al., 2007) and flower damage (Rotolo et al., 2018), but most importantly for infection during on-vine ripening (Pertot et al., 2017) and in postharvest stored bunches (De Simone et al., 2020), which lead to high production losses (Agudelo-Romero et al., 2015).

Botrytis cinerea is a necrotrophic pathogen which is unable to obtain nutrients from living tissues and uses a pathogenic mechanism which includes the production of toxins and hydrolytic enzymes to destroy plant leaves or berries tissues (González et al., 2015). After the initial contact with the host, two distinct phases occur. The early phase (i.e., until 36 hours post inoculation) is characterized by the formation of local infection foci without spreading, facilitated by fungal compounds that help in rapid killing of host cells. Dead cells remain viable due to the suppression of autophagic cell death that prevents activation of typical self-killing. This phase usually culminates in the killing of a sufficient number of host cells to create a region of dead tissue in which fungal biomass accumulates prior to the transition to intermediate and then

* Corresponding author.
E-mail address: stefano.brizzolara@santannapisa.it (S. Brizzolara).
§ These authors have contributed equally to this work and share first authorship

https://doi.org/10.1016/j.stress.2025.100803
Received 13 December 2024; Received in revised form 20 February 2025; Accepted 11 March 2025
Available online 11 March 2025

late infection phases. Fungal survival depends in part on antiapoptotic machinery that prevents the death of the entire fungal biomass, being the success of the infection strictly dependent on the balance between plant and fungal cell death (Bi et al., 2023).

Grey mold symptoms vary depending on the host and the plant part attacked, and include (i) grey to brown discoloration, (ii) water soaking, and (iii) fungal structures (e.g. mycelium and conidia) growing on the surface of the affected tissue and restricted lesions (Droby and Lichter, 2007, Blanco-Ulate et al., 2016). In grapes, the susceptibility to *B. cinerea* increases during berry ripening, and infection can be conveyed by animals (e.g. birds, insects, rodents), mechanical injury, sunscald, and rain cracking (Coertze and Holz, 2002). Rainfall or ineffective insect management in the vineyard can trigger fungal outbreaks, subsequently affecting the quality of grape bunches after harvest, especially when there is a long period of dehydration.

Although withering occurs at relatively low temperatures (10–15 °C), *B. cinerea* is a major issue during the postharvest period due to its ability to grow at temperatures just above freezing (Droby and Lichter, 2007). Considering specifically wine grapes, many winegrowers in Italy that produce special wines such as Amarone and Ripasso (Valpolicella, Veneto) and Vin Santo (Tuscany) partially dehydrate harvested berries (Mencarelli and Tonutti, 2013). Dehydration can reach 30% weight loss or even more, resulting in grapes that are rich in sugar and aroma compounds which contribute to the unique characteristics of the wines. In addition to their concentration, the composition of dehydrating berries can be affected by processes related to polyphenol and volatile metabolisms, the structure of cell wall polysaccharides, and the activity of enzymes involved in anaerobic respiration (Costantini et al., 2006; Rizzini et al., 2009; Zoccatelli et al., 2013; Sanmartin et al., 2021).

How berries respond to the dehydration protocol depends both on environmental parameters and endogenous factors, such as berry morphology and genotype (Zamboni et al., 2008; Zenoni et al., 2016; Brizzolara et al., 2020). The intensity of biochemical and molecular modifications is correlated with the water loss rate (Zenoni et al., 2020). In addition, extensive modulation of gene expression in berry tissues during prolonged postharvest dehydration has been described (Bonghi et al., 2012; Zenoni et al., 2016).

Since the dehydration rate is mainly dependent on temperature and relative humidity, an appropriate combination is needed to obtain the desired withering kinetics. Most dehydration facilities are therefore equipped with dehumidification systems and air fans that are extremely energy-demanding and have a high economic and environmental impact. Maintaining a high relative humidity and, hence, reducing both the costs and the impact of operating dehydration facilities, is based on finding practical solutions that are effective at controlling postharvest decay. In fact, the long duration of the dehydration process (up to three/four months, depending on the grape variety and wine type) increases the risk of developing fungal diseases such as grey mold by *B. cinerea*.

The high relative humidity and the lack of ventilation inside the dehydration facilities are the most favorable conditions for the diffusion of grey mold. Internal berry disintegration is typically followed by the appearance of aerial mycelium and sporulation which serves as an inoculum source for subsequent infection. The decaying berry itself infects neighbouring berries by forming 'nests' which can eventually spread over the entire bunch. Infection can also develop from the pedicel-berry interface. Fewer typical decay symptoms may be observed as lesions with clear boundaries in limited areas (Droby and Lichter, 2007).

Like other fruits, grape berries naturally emit aromatic molecules known as volatile organic compounds (VOCs). Abiotic or biotic stress can alter the aromatic profile of fruits. The production/modulation of specific volatile molecules has been found to be modified by *B. cinerea* infection in grape berries. Molecules such as trans-2-hexenal appear to decrease with infection (Schueuermann et al., 2019; Santos et al., 2022), while compounds such as several acetate esters (e.g., 2-phenyethyl acetate, hexyl acetate, and isoamyl acetate) increase (Santos et al., 2022). These acetate esters are normally absent or at very low levels in berries (grape juice), while they are found at high levels in wine as they are produced by yeasts during fermentation (Dennis et al., 2012; Boss et al., 2015).

Other molecules that are positively associated with these necrotrophic pathogens are linked to other chemical classes, such as alcohols derived from C6 VOC metabolism (e.g., hexanol), mono- and sesquiterpenes (e.g., pinene, terpinene, copaene, caryophyllene), and others (e.g., methyl salicylate). Similar results have also been reported after studying the effects of *B. cinerea* infection in other species, such as tomato (Jansen et al., 2009, 2010). Some of these molecules are also produced by, or in response to, other pathogens, such as *Fusarium* spp. in cereal crops (Piesik et al., 2011; Buśko et al., 2014).

Compounds such as 3-octanone, 3-octanol, 1,5-dimethylnaphthalene and 1,5-dimethyltetraline have been reported as potential markers for early infection diagnosis, as their accumulation was found to be significantly high in infected grapes in the first seven days after artificial inoculation (Jiang et al., 2023). All these published papers on the specific modifications of the grape berry VOC profile in response to *B. cinerea* infection have collected data from processed (freezing and/or grinding) samples. Thus, identifying the volatile markers of *B. cinerea* that characterise the early stages of the infection in intact grape berries represents an innovative step and is of paramount importance for the development of sensors for a possible "*in situ*" monitoring. This could thus potentially lead to an early diagnosis of the fungal infection. New VOC sensors have been developed in several fields, including the food industry (Cova et al., 2022). Metal-oxide (MOX) based sensors monitor specific compounds/chemical classes of volatiles in the air.

Extending the current knowledge on the volatile markers of *B. cinerea* could lead to the development of specific MOX sensors which could be used for the early diagnosis of *B. cinerea* in dehydration facilities, and potentially for other important applications (e.g., in-field diagnosis). An early diagnosis of the infection in the final stages of dehydration could allow wineries to make rapid decisions regarding the vinification process. Such sensors would also help to reduce spoilage via targeted and timely interventions. In terms of sustainability, it could also reduce the energy consumption of the artificial control of the environmental parameters in dehydration facilities.

2. Materials and methods

2.1. Berry dehydration protocol and sampling

Grape bunches of Corvina and Sangiovese cultivars were manually harvested at commercial ripening in the 2023 growing season, following a visual assessment of their integrity. The bunches (about 5–6 kg) were laid in one layer inside perforated plastic trays (40×60×15 cm each) that were placed in an experimental dehydration chamber for the withering process under thermo-hygrometric controlled conditions of 12 °C and 60% relative humidity. Weight loss (WL) was monitored by real-time floor scales (Sordato Holding s.r.l, Verona, Italy). Berry samples were collected at harvest (fresh berries, T0) and after 40 days of withering (T40). Sound berries were selected based on the homogeneity of their weight, and the pedicel was left attached to avoid increasing intra-sample variability. Technological parameters were measured at both sampling points (Table 1). Due to the different characteristics of the two cultivars, the second sampling point (T40) was characterized by different dehydration stages for the two cultivars: 30 and 15% of WL for Sangiovese and Corvina, respectively (Figure S1 and S2, Supplementary material).

2.2. Artificial inoculation with Botrytis cinerea

Botrytis cinerea (strain 8335, fungal collection of the Department of Agriculture, Food and Environment, University of Pisa) was grown on Potato Dextrose Agar (PDA, 42 g L^{-1}, BioLife, Milano, Italy) added with

Table 1
Weight (g), total soluble solids (TSS, °Brix), pH and titratable acidity (TA, g/L) of Sangiovese and Corvina grapes. Values (± SD) were collected at harvest (T0) and after 40 days of dehydration (T40).

Cultivar	Berry weight		TSS		pH		TA	
	T0	T40	T0	T40	T0	T40	T0	T40
Sangiovese	1.86 ± 0.06	1.38 ± 0.06	23.6 ± 0.40	29.5 ± 1.45	3.5 ± 0.08	3.7 ± 0.04	6.1 ± 0.50	8.1 ± 0.00
Corvina	2.22 ± 0.07	1.94 ± 0.13	23.2 ± 0.25	26.2 ± 0.28	3.3 ± 0.02	3.3 ± 0.03	5.6 ± 0.08	5.8 ± 0.45

streptomycin sulfate (0.1 g L^{-1}) in Petri dishes (∅ 6 cm) and kept for seven days at 23 °C and 12 h photoperiod. A spore suspension of *B. cinerea* was obtained scratching conidia in a sterile solution of sucrose (2%, w/v) and yeast extract (0.05%, w/v) in water, prepared in flask (0.5 L), and maintained in orbital homogenizer (711 CT, Asal Milano) set at 150 rpm for 48 h at room temperature. The concentration was adjusted to 10^5 conidia mL^{-1} through an hemocytometer (Henneberg-Sander, Giessen Lützellinden, Germany) prior inoculation, according to Modesti et al. (2024). The sterile solution was used for the mock inoculation. Control and inoculated samples collected at harvest and after 40 days of dehydration from both cultivars were placed in different grids depending on the treatment. The following samples have been considered: i) sound intact berries (CTRL), ii) berries artificially inoculated with 10 μL of a suspension containing 10^5 spores mL^{-1} of *B. cinerea* (A_Inoculum), and iii) mock inoculated berries by using the same volume of growth medium (CTRL_Wound; Figure S3, Supplementary material). Since a small wound must be applied in inoculated berries to allow for the infection, wounded samples (CTRL_Wound) have been included as additional control samples to discriminate between variations of the VOCs profile solely induced by the wound and those induced by the presence of infection. Samples were incubated at the same temperature set in the dehydration chambers (12 °C) to mimicking the same evolution/timing of the natural infections taking place in this specific type of environment. After 1, 3 and 5 days of incubation, two berries were placed in clear screw vials of 40 mL for volatile profiling.

2.3. SPME-GC-MS analysis of berries VOCs profile

VOCs analysis was performed using gas chromatographer (GC, Clarus 680) coupled with mass spectrometry (MS, Clarus 600 S) (PerkinElmer®, Waltham, MA, US) using solid-phase microextraction (SPME) technique. Samples incubation and VOCs extraction were done simultaneously into clear screw cap 40 mL vials (Agilent Technologies, Santa Clara, CA, US). VOCs collection has been performed at 35 °C for 3 hours placing the vials in a thermostatic water bath (Julabo SW 1, JULABO GmbH, Seelbach, Germany) and inserting a SPME fiber (50/30 μm DVB/CAR/PDMS coating, 2 cm, Supelco Inc., Bellefonte, PA, US) through the septa of the vial caps. After extraction, VOCs were thermally desorbed into the GC injector (PSSI, splitless injection) heated at 250 °C for 5 min. VOCs separation was performed using a SUPELCOWAX™ column (L×I.D. 60 m×0.25 mm, 0.5 μm film thickness, Sigma-Aldrich, St. Louis and Burlington, MA, US), and helium was used as carrier gas at a constant flow rate of 1 mL/min. The GC oven was preheated at 40 °C (hold for 5 min) and then programmed as follow: 120 °C at a rate of 10 °C/min, hold for 5 min; 180 °C at a rate of 2 °C/min, hold for 2 min; 230 °C at a rate of 10 °C/min and hold for 5 min, with a total runtime of 62 min. AMDIS (Automatic Mass Spectral Deconvolution and Identification System) software was used to facilitate compounds identification comparing deconvoluted peak spectra to a reference database (National Institute of Standards and Technology, NIST). Retention index was calculated for each compound and compared with the indexes reported in the libraries for the same column type. Only compounds possessing 80%, or more, of matching were considered. The quantification of the different identified VOCs was carried out by calculating their relative intensity, which was obtained calculating the ratio of the area of each specific peak present in a sample on the sum of the areas of all the compounds identified in that specific chromatogram. This approach stands for a normalized assessment of the abundance of individual compounds within the chromatographic data which allows for avoiding variation due to fiber decay.

2.4. Statistical analysis

Data analysis was performed on six biological replicates using JMP® (18.0.0 2024, JMP Statistical Discovery LLC, Cary, NC, US). One-way ANOVA and mean comparison with least significant difference (LSD) post-hoc test was used to compare different samples. Kruskal-Wallis tests was conducted on samples not meeting ANOVA assumptions, while Wilcoxon test was used as post-hoc test to compare their means. Partial least squares discriminant analysis (PLS-DA) models have been created to address two important goals. A first PLS-DA model has been created using only CTRL samples to investigate differences in VOC profiles induced by the dehydration process. To build this model VOCs commonly identified in both fresh and dehydrated grapes have been used as predictor variables, while cultivar and dehydration treatment have been employed as response variables. The other PLS-DA models, built separately for each cultivar, were created to identify potential markers of early stages of infection. To construct these models the identified VOCs were employed as predictor variables and treatment was used as response variable. All the created PLS-DA models were validated through cross-validation process and variable importance in projection scores (VIPs), with a threshold set at 0.8, have been used to filter important features of interest. Univariate analysis results were plotted using R studio (R version 4.3.2).

3. Results

The profiling of VOCs from intact grape berries that had been artificially inoculated produced different results depending on the incubation time (1, 3 and 5 days after inoculation), the duration of the berry dehydration (0 and 40 days) and the specific cultivar (Sangiovese and Corvina).

A total of 45 and 74 VOCs were identified in fresh and dehydrated control samples, respectively, in both analysed cultivars (Table S1, Supplementary material). The molecules identified belong to different chemical classes, including aldehydes, alcohols, ketones, esters, organic acids and terpenes. Among these compounds, 31 molecules were identified only in the dehydrated samples, while 2 compounds were detected exclusively in the fresh samples.

To better understand the differences in VOC emissions between the two cultivars at different stages of dehydration, CTRL samples from both fresh and partially dehydrated grape samples from each cultivar were analysed. Fig. 1 displays the biplot from a PLS-DA performed using only the common compounds identified in both fresh and dehydrated CTRL samples. The first two factors of the model explained about 56% of the dataset variability and managed effectively to separate sample types. Compounds such as ethanol, isobutanol, 2-hexen-1-ol, phenylethanol, nonanal and pyroterebic acid were present at higher levels in fresh samples than in dehydrated berries. On the other hand, compounds such as pentanol, 1-penten-3-ol, hexanol, heptanol, 1-octen-3-ol and ethyl dodecanoate were located closer to the dehydrated berries, thus revealing higher amounts in these specific samples. In addition, the majority of the terpenes detected appeared to be strongly associated with dehydrated samples from the Corvina cultivar. However, most of

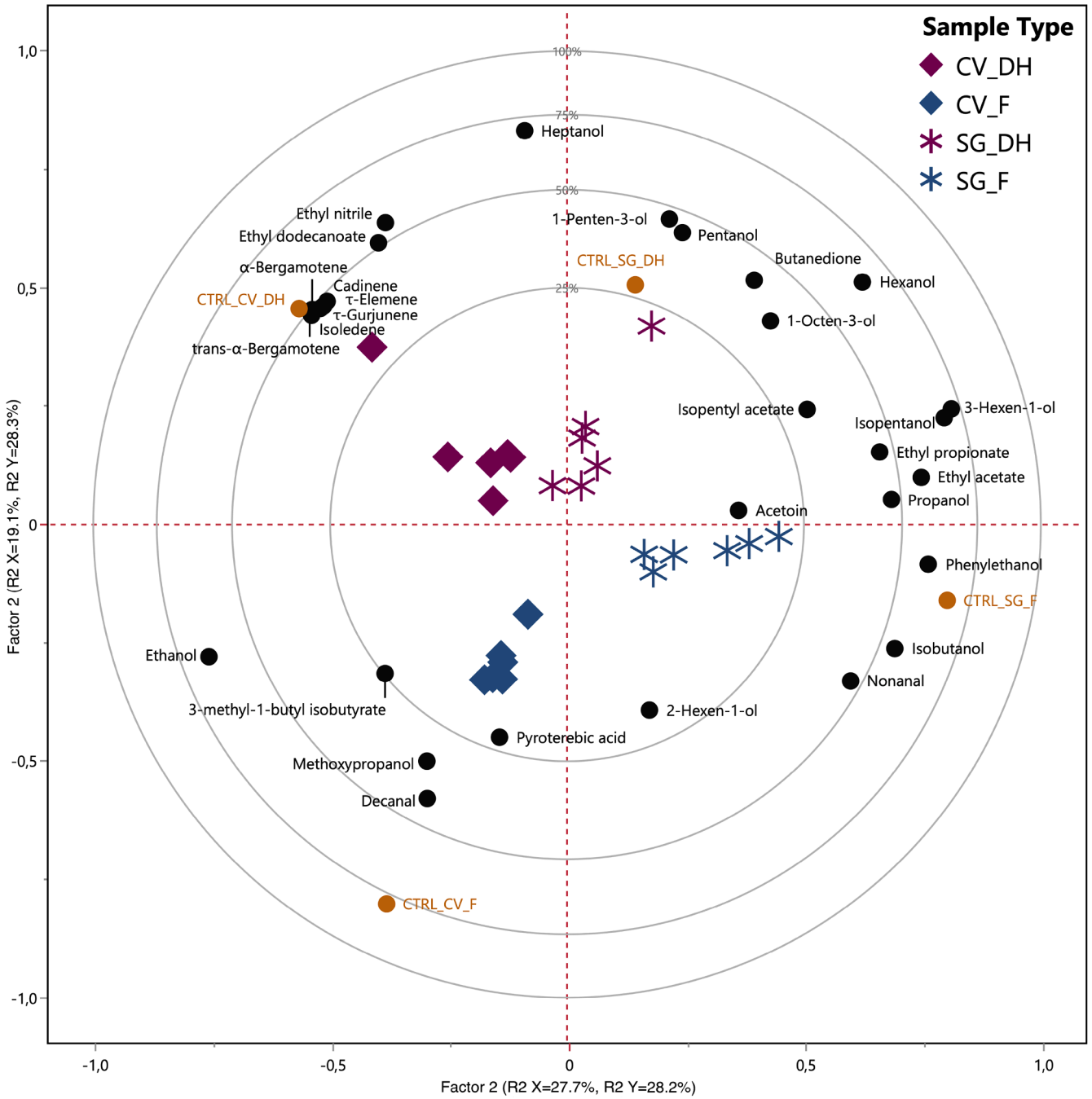


Fig. 1. Partial least squares discriminant analysis (PLS-DA) performed on CTRL samples from both wine grape cultivars. VOCs commonly identified in fresh and dehydrated samples have been used as predictor variables, while cultivar and dehydration treatment have been employed as response variables. Grapes from Corvina cultivar have been tagged with "CV", whereas "SG" tag has been used for Sangiovese grapes. Blue-green and purple colors have been used to depict fresh (CTRL_CV_F and CTRL_SG_F) and dehydrated (CTRL_CV_DH and CTRL_SG_DH) samples, respectively. Variable importance in projection scores (VIPs) have been employed to filter important variables contributing to samples clustering.

the detected VOCs tended to show higher amounts in the Sangiovese CTRL berries.

3.1. Fresh berries

In fresh berries artificially inoculated with *B. cinerea* and analysed one and three days after inoculation, samples from the three different experimental groups (CTRL, A_Inoculum and CTRL_Wound) showed very similar VOC profiles. Only isobutanol showed important differences in the accumulation pattern of Corvina samples analysed after 1 day from the inoculum, with significantly higher values in CTRL samples compared to CTRL_Wound grapes, while A_Inoculum showed no significant differences compared to the other samples (Figure S4, Supplementary material).

Fig. 2 shows a biplot of a PLS-DA model created analysing fresh samples of Corvina. The first two factors of the model explained approximately 56% of the total variability present in the dataset. The score plot shows a relatively clear samples clustering, with the three sample groups appearing separated from each other. Compounds as 1-penten-3-ol, 1-octen-3-ol, 2-hexenal, hexanol, 2-hexen-1-ol, pentanol and pyroterebic acid seem to be associated with A_Inoculum samples, while ethyl propionate and ethyl acetate, belonging to the chemical class

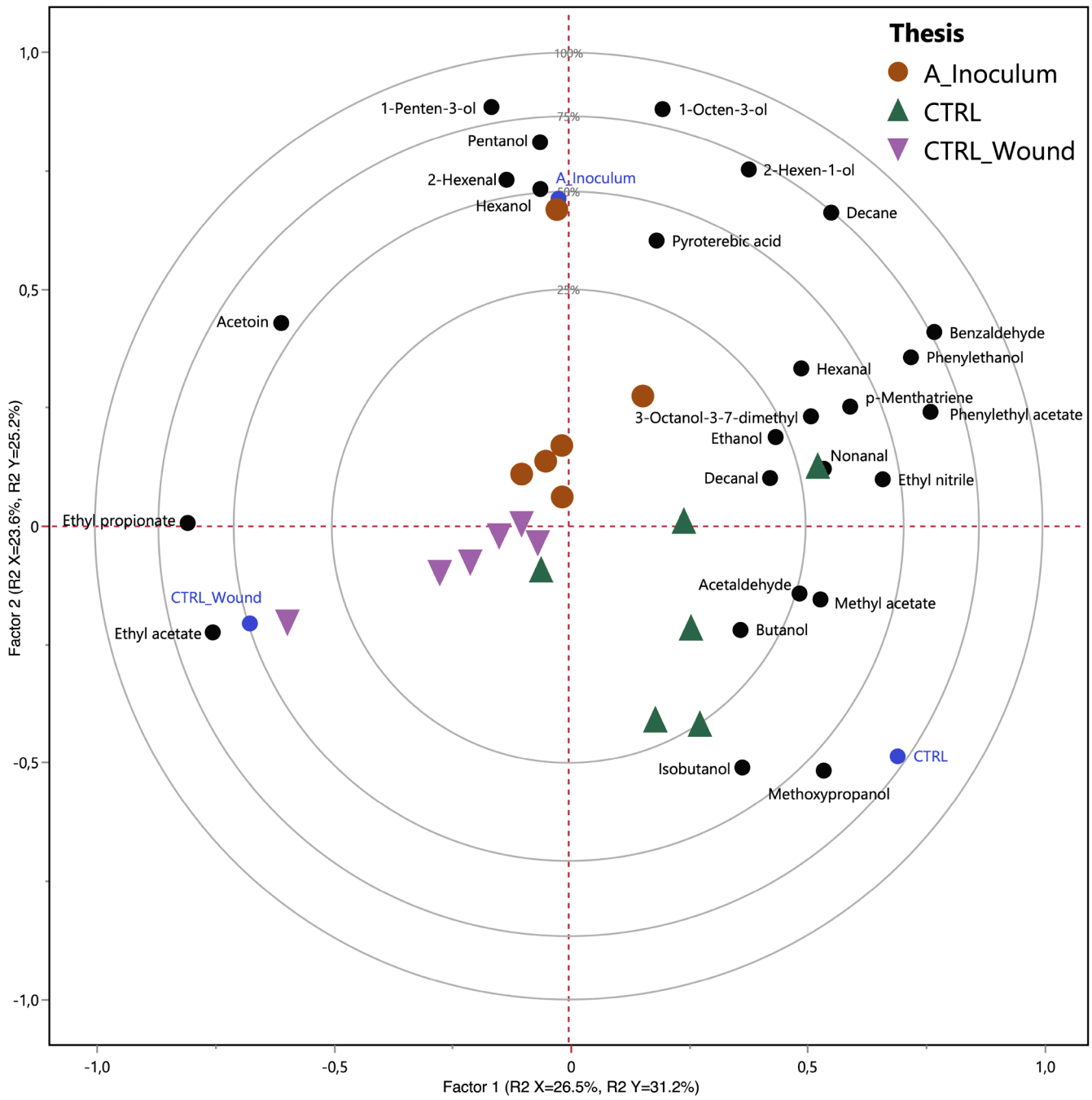


Fig. 2. Partial least squares discriminant analysis (PLS-DA) performed on fresh samples of Corvina after 5 days from the inoculation. Treatment has been used as response variable, while VOCs detected has been used as predictor variables. Green, purple and red colors have been used to depict CTRL, CTRL_Wound and A_Inoculum samples, respectively. Variable importance in projection scores (VIPs) have been employed to filter important variables contributing to samples clustering.

of ethyl esters, appear to sit closer to CTRL_Wound samples. On the other hand, compounds as acetaldehyde, methyl acetate, butanol, isobutanol and methoxypropanol seem to be correlated with CTRL samples.

The results of univariate statistical analysis of the same dataset confirmed that 1-penten-3-ol and 1-octen-3-ol accumulate at significantly higher levels in inoculated samples (Fig. 3). In addition, 2-hexenal showed important differences among samples, despite significantly higher levels being recorded only in the comparison between A_Inoculum and CTRL samples. Two other volatiles, ethyl acetate and ethyl propionate, showed significant differences among samples. However, while ethyl propionate revealed higher accumulation levels in both A_Inoculum and CTRL_Wound samples compared to CTRL berries, ethyl acetate showed differences only considering CTRL_Wound samples, which possess higher level of this ester compared to the other grape samples.

Sangiovese grapes that had been set aside for analysis on fresh berries experienced mechanical stress during transport and manipulation. This may have altered their metabolism even before the inoculation, resulting in non-significant variations among samples even five days after inoculation (data not shown).

3.2. *Partially dehydrated berries*

Similarly to what reported for fresh berries, also VOCs analysis of

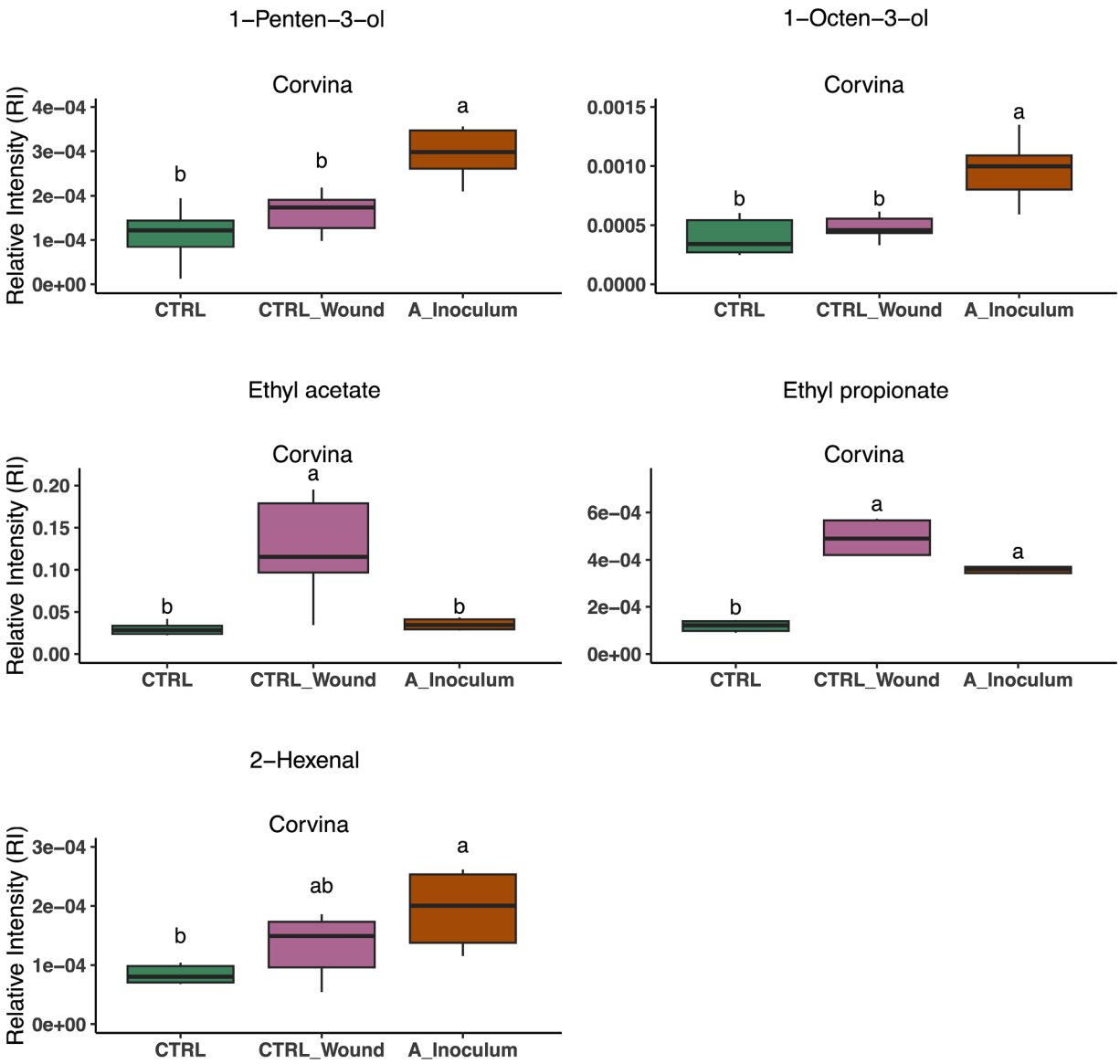


Fig. 3. Accumulation pattern of 1-penten-3-ol, 1-octen-3-ol, ethyl acetate, ethyl propionate and 2-hexenal in the different fresh samples after 5 days from inoculation. Green, purple and red colors have been used to depict CTRL, CTRL_Wound, and A_Inoculum samples, respectively. Different letters indicate statistically significant differences between groups (Kruskal-Wallis, Wilcoxon test *p-value* $\leq$ *0.05* or ANOVA, LSD test *p-value* $\leq$ *0.05*).

partially dehydrated grapes performed one and three days after inoculation showed very similar profiles among different samples. The only exception is 2-ethyl hexanol, that revealed significant differences in the accumulation pattern of Sangiovese grapes analysed after three days from the inoculum. This volatile showed significantly higher values in CTRL samples compared to both CTRL_Wound and A_Inoculum grapes, which shared similar levels (Figure S5, Supplementary material). These results suggest a delay of a few days, during which artificial inoculation did not appear to induce any important difference in VOC levels among the samples. In fact, as observed in fresh berries, significant differences were only detected after 5 days post-inoculation, a trend consistent across both cultivars. Fig. 4 shows two PLS-DA models that were created by separately analysing the datasets obtained for the two different cultivars. Both models revealed relatively high values of explained variability within each dataset, approximately 67% for Sangiovese (Fig. 4A) and 57% for Corvina (Fig. 4B). Moreover, relatively clear clusters of berry samples are shown in the model score plots, with the three sample groups appearing separate from each other. This separation was clearer for Sangiovese cultivar than for Corvina, which also showed a more scattered sample distribution and a partial overlap of the different groups in the score plot (Fig. 4B). Some results were common for both models, with a subset of specific molecules being closer to each of the three different sample groups.

Considering CTRL samples, both cultivars presented higher levels of acetaldehyde, ethanol, isobutanol, butyl carbitol and carvone. On the other hand, ethyl acetate and ethyl propionate were associated with the CTRL_Wound samples in both cultivars. Finally, the content of 1-penten-3-ol, hexanol, 2-hexen-1-ol, 3-hexen-1-ol, nonanal and methyl acetate appeared to be higher in the A_Inoculum samples in both grape cultivars. Interestingly, a clear cluster of most of the detected volatile terpenes was observed in both models. This chemical class appeared to be more closely associated with the CTRL_Wound and CTRL samples in Corvina and Sangiovese cultivars, respectively.

To better clarify the relationships between the VOCs measured and the different sets of samples, data were analysed using univariate statistical analysis. Fig. 5 shows the accumulation patterns of the few compounds with statistically significant results. A higher concentration of these compounds was generally found in A_inoculum grapes

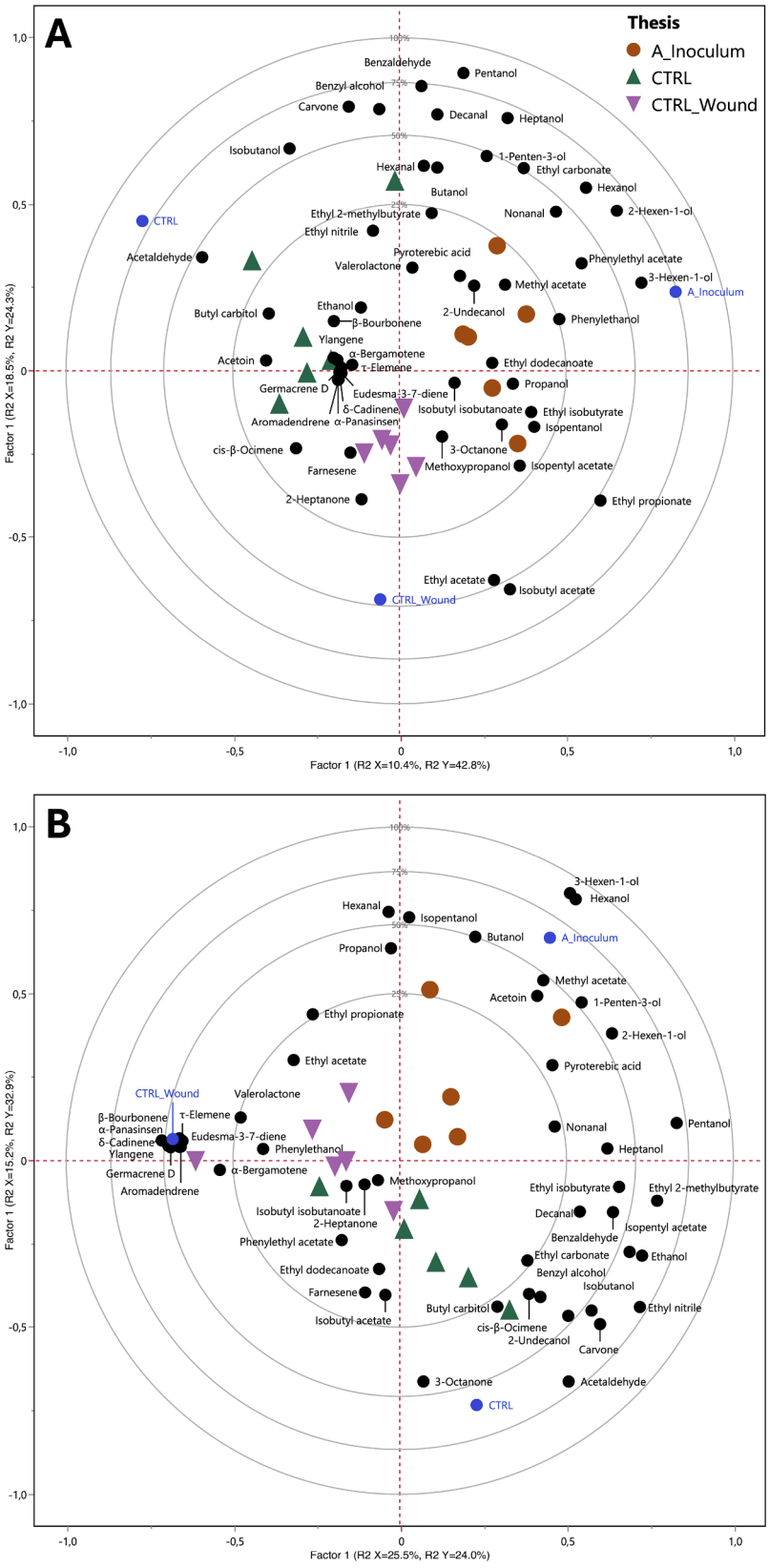


Fig. 4. Partial least squares discriminant analysis (PLS-DA) performed on partially dehydrated samples after 5 days from the inoculation. A specific PLS-DA model has been created for each cultivar, Sangiovese (A) and Corvina (B), using treatment as response variable and the VOCs detected in partially dehydrated berries as predictor variables. Green, purple and red colors have been used to depict CTRL, CTRL_Wound and A_Inoculum samples, respectively. Variable importance in projection scores (VIPs) have been employed to filter important variables contributing to samples clustering.

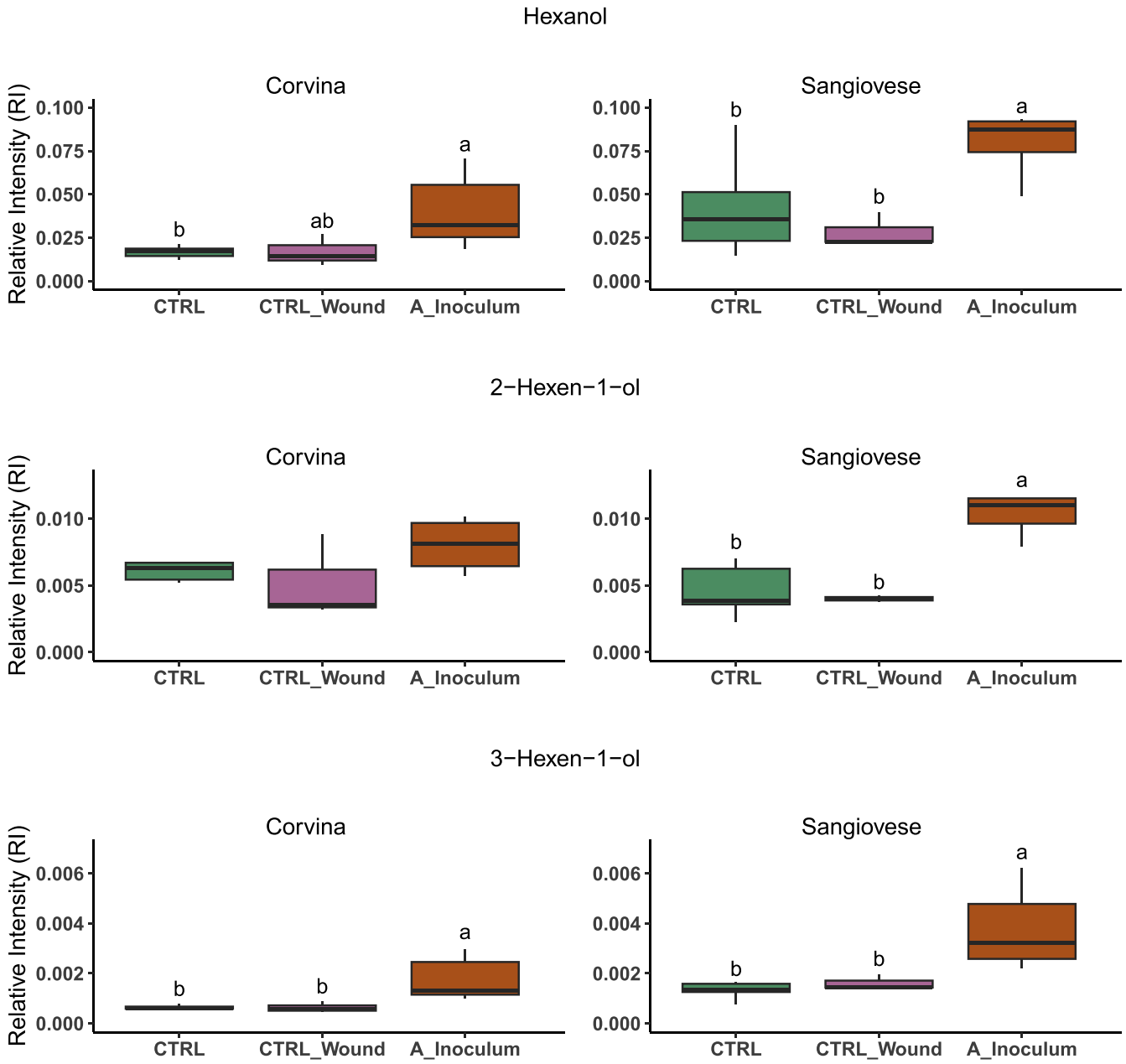


Fig. 5. Accumulation pattern of hexanol, 2-hexen-1-ol and 3-hexen-1-ol in the different partially dehydrated samples after 5 days from inoculation. Green, purple and red colors have been used to depict CTRL, CTRL_Wound and A_Inoculum, respectively. Different letters indicate statistically significant differences between groups (Kruskal-Wallis, Wilcoxon test *p-value* $\leq$ *0.05* or ANOVA, LSD test *p-value* $\leq$ *0.05*).

compared to the other samples, which could be directly linked to *B. cinerea* infection. The potential infection markers identified were hexanol, 2-hexen-1-ol and 3-hexen-1-ol, all C6 volatile alcohols, with similar accumulation patterns across the two cultivars. However, only 3-hexen-1-ol was significantly higher in A_inoculum samples compared to CTRL and CTRL_wound samples in both cultivars. 2-hexen-1-ol and hexanol followed the same trend in Sangiovese samples. In Corvina samples, hexanol showed significant differences between A_inoculum and CTRL, with intermediate levels in CTRL_wound samples. 2-hexen-1-ol did not show significant differences in Corvina.

Fig. 6 reports two additional compounds that showed significant differences among samples, but a significant induction or reduction did not appear to be as clearly associated with *B. cinerea* inoculation. Despite always showing higher levels in A_Inoculum berries than in the other samples in both cultivars, nonanal showed significant differences only comparing A_inoculum samples with CTRL_wound berries. Acetaldehyde showed significant differences between the CTRL and A_inoculum samples only in the Sangiovese cultivar, with the CTRL berries showing higher levels than the other samples.

4. Discussion and conclusions

The results of this work confirmed that specific VOCs can be identified as potential markers of *B. cinerea* infection by analysing intact grape berries artificially inoculated with the fungus. This is of particular interest for the potential development of VOC sensors for the early diagnosis of *B. cinerea* infection in intact grape bunches. The VOC profiling results of both fresh and partially dehydrated samples presented distinct VOC patterns after five days from the inoculation. The potential volatile markers identified at this sampling time can therefore still be correlated with the early phases of *B. cinerea* infection.

The VOC profile clearly changed with dehydration, with 32 volatiles

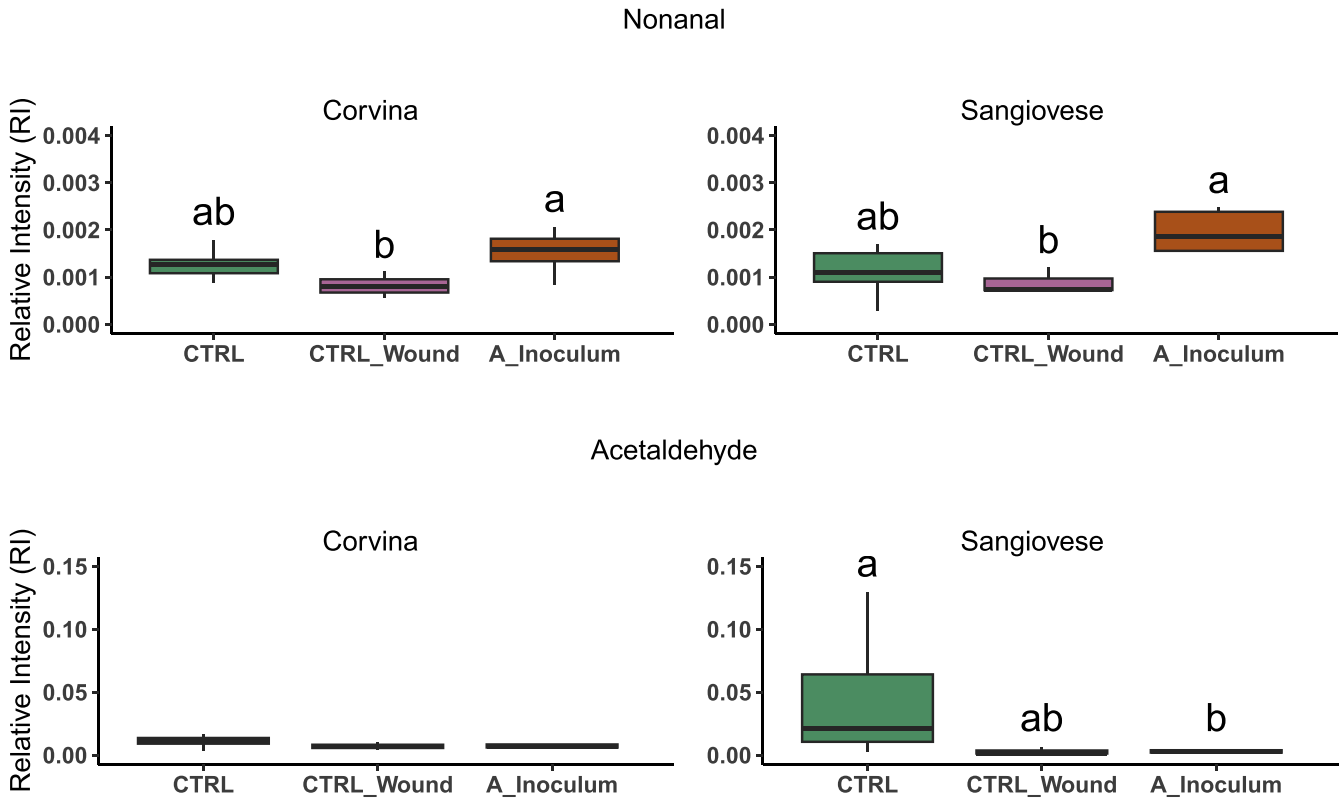


Fig. 6. Accumulation pattern of nonanal and acetaldehyde in the different partially dehydrated samples after 5 days from inoculation. Green, purple and red colors have been used to depict CTRL, CTRL_Wound and A_Inoculum, respectively. Different letters indicate statistically significant differences between groups (Kruskal-Wallis, Wilcoxon test *p-value* $\leq$ *0.05* or ANOVA, LSD test *p-value* $\leq$ *0.05*).

that were not detected in the fresh samples (Table S1). Among the VOCs commonly identified in fresh and dehydrated samples, the accumulation patterns of different chemical classes appeared to be altered by dehydration. Sanmartin et al. (2021) reported an accumulation of aldehydes, alcohols, esters, lactones, and terpenes during the dehydration process. This behaviour was partially confirmed in our study. In fact, terpenes clearly increased with dehydration, especially in the Corvina samples, as well as some esters (e.g., ethyl dodecanoate) and alcohols (e.g., pentanol). A clear effect of the genotype has also been identified on VOC profiles, with the two analysed cultivars appearing clearly separated in the score plot (Fig. 1). However, the observed pattern of VOC accumulation may also be influenced by the different dehydration levels that the two cultivars reached at the time of the sampling.

The VOCs that have been shown to be most closely associated with the infection mainly belong to the green leaf volatile (GLV) class, which originate from the lipoxygenase (LOX) pathway and are usually produced in plant tissues in response to biotic and/or abiotic stress (Pellegrini et al., 2012).

The analysis of fresh samples of Corvina revealed that the levels of VOCs, such as 2-hexenal, 1-penten-3-ol, 1-octen-3-ol and ethyl propionate, were significantly higher in A_Inoculum compared to CTRL berries (Fig. 3). In strawberries, 2-hexenal has been shown to act as a *B. cinerea* growth inhibitor (Myung, 2005; Wakai et al., 2019). The accumulation of this aldehyde in the initial days after berry artificial inoculation could thus be part of a defence mechanism induced in grape berries after infection. However, our data revealed intermediate levels of 2-hexenal in CTRL_Wound samples, indicating an involvement of this volatile also in the response to wounding as also suggested by Wang et al. (2015).

Considering 1-penten-3-ol, this C5 alcohol has not yet been reported in the literature as being directly associated with *B. cinerea* infection. However, its level has been shown to increase in plant tissues subjected to different types of damage, including pathogen attack (Scutareanu et al., 1997; Fisher et al., 2003). Moreover, the application of 1-penten-3-ol on maize was shown to increase resistance to *Colletotrichum graminicola* (Gorman et al., 2021). 2-hexenal and 1-penten-3-ol are produced early in the LOX pathway, thus suggesting they play an important role in the very first plant response mechanisms. The first volatile belongs to the GLV class, while 1-penten-3-ol belongs to the pentyl leaf volatile (PLV) class, which derive from another branch of the LOX pathway but, structurally, are very similar to GLVs. Because of these similarities, PLVs are often co-emitted with GLVs in response to various abiotic and biotic stresses in many plant species (Fall et al., 2001; Heiden et al., 2003; Jardine et al., 2012; He et al., 2020).

1-octen-3-ol has often been reported as associated with *B. cinerea* infection (Schueuermann et al., 2019; Jiang et al., 2023). The results of our study also confirmed this in intact inoculated berries, but only in fresh samples (Fig. 3). In partially dehydrated berries 1-octen-3-ol levels did not significantly differ from the artificial inoculation. Additionally, ethyl propionate presented higher levels in the inoculated fresh samples, but it appeared to have similar levels in both A_Inoculum and CTRL_Wound berries. This ester has been reported to highlight *Aspergillus niger* infection in the grape juice of Chardonnay cv (Schueuermann et al., 2019).

Regarding to the partially dehydrated samples, VOCs such as hexanol, 2-hexen-1-ol and 3-hexen-1-ol, which all derive from the LOX pathway, were identified as the best potential markers of *B. cinerea* infection and were significantly higher in the inoculated berries of both cultivars (Fig. 5). In contrast, a previous study on grapes (Santos et al., 2022) reported a reduction in C6 VOC emissions in infected samples. However, in this Santos's study, the grape samples were ground prior to VOC analysis and the study was conducted on grapes that had been artificially inoculated in the field and then harvested with the infection at an advanced stage. In fact, after an entire season on-vine, the infected

berries were described as necrotized and desiccated. Another study reported a significant decrease in total free GLV (including C6) emissions in grapes during postharvest controlled dehydration (Piombino et al., 2022). This is reasonable since C6 VOCs such as 2-hexenal, hexanol, 2-hexen-1-ol and 3-hexen-1-ol are produced almost instantly from plant tissues in response to stress/damage, and a decrease in the emission of these compounds may occur over time.

Our results did not reveal a clear trend in the accumulation pattern of C6 volatiles comparing fresh and dehydrated CTRL samples, although this VOC class generally appeared to have higher levels in Sangiovese cultivar (Fig. 1). The majority of these molecules showed similar levels between the fresh and dehydrated samples. The only exception was 2-hexen-1-ol which was more associated with the fresh samples and, therefore, confirmed the hypothesis of a decrease overtime in C6 VOC emissions during dehydration. Combined with the fact that in sound dehydrating berries these compounds were found to decrease in line with the dehydration time (Piombino et al., 2022), these results could reinforce the hypothesis that the significant increase observed in some GLVs in intact grape berries a few days after inoculation is related to the infection.

Interestingly, 1-penten-3-ol seems to be associated with infection in both fresh and partially dehydrated samples. However, in fresh Corvina berries 1-penten-3-ol was clearly correlated with A_Inoculum samples in the reported PLS-DA model and univariate statistics confirmed the significance of this trend (Figs. 2 and 3). On the other hand, in the dehydrated berries of both cultivars this trend was only corroborated by a good correlation in the PLS-DA model, which was not supported by statistical significance (Fig. 4). Overall, these results seem to confirm 1-penten-3-ol behaviour in response to pathogen attack, as already reported, and suggest the potential involvement of this PLV in the grape response to *B. cinerea* infection.

Other detected VOCs previously reported in the literature as potential markers of *B. cinerea* infection, namely pentanol, isopentanol and phenyl ethanol (Santos et al., 2022), showed a positive correlation with partially dehydrated intact berries that had been artificially inoculated with *B. cinerea* (Fig. 4). For isopentanol and phenyl ethanol this positive correlation is true only for Corvina and Sangiovese grapes, respectively. Moreover, pentanol also showed a very good correlation with fresh A_Inoculum samples (Fig. 2). However, these results were not supported by significance when performing univariate statistical analysis.

Different trends in VOC emissions in response to infection can be due to several factors, such as the specific genotype, the harvesting season, the dehydration levels of the grapes and/or the employed method of VOC profiling. Although it is common practice to conduct SPME-GC-MS analysis on frozen ground grape samples (Santos et al., 2022; Jiang et al, 2023), or on purees obtained by adding salt buffers to frozen grape tissue, the aim of this study was to identify VOCs that had been specifically emitted after an infection induced in intact grape berries. In addition, the freeze-thawing of plant tissues prior to VOC profiling has been reported to strongly impact the emissions of GLVs and PLVs (Gorman et al., 2021). These aspects need to be carefully considered when developing VOC sensors to be applied in dehydration facilities directly on intact grape bunches.

Overall, these preliminary results contribute to further the basic knowledge on grey mold related VOCs. We believe that our findings may pave the way for the development of effective MOX sensors capable of an early diagnosis of infection in dehydration facilities during grape dehydration.

CRediT authorship contribution statement

Pietro Emilio Nepi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Claudia Pisuttu:** Writing – review & editing, Methodology. **Cristina Nali:** Writing – review & editing, Supervision. **Elisa Pellegrini:** Writing – review & editing. **Ron Shmuleviz:** Writing – review & editing. **Stefano Brizzolara:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Pietro Tonutti:** Writing – review & editing, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This work was financially supported by the project "Ozone application for low input and sensor-monitored postharvest dehydration of wine grapes" – 2022RAY28P – CUP J53D23010400006 (PRIN 2022), funded by the Italian Ministry of University and Research (MUR) to P. Tonutti.

Statement of AI Usage

The authors declare that AI was exclusively used to check the English of the paper, not to generate any text.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2025.100803.

Data availability

Data will be made available on request.

References

Agudelo-Romero, P., Erban, A., Rego, C., Carbonell-Bejerano, P., Nascimento, T., Sousa, L., Fortes, A.M., 2015. Transcriptome and metabolome reprogramming in Vitis vinifera cv. Trincadeira berries upon infection with Botrytis cinerea. J. Exp. Bot. 66 (7), 1769–1785. https://doi.org/10.1093/jxb/eru517.

Bi, K., Liang, Y., Mengiste, T., Sharon, A., 2023. Killing softly: a roadmap of *Botrytis cinerea* pathogenicity. Trends. Plant Sci. 28 (2), 211–222. https://doi.org/10.1016/j.tplants.2022.08.024.

Blanco-Ulate, B., Vincenti, E., Cantu, D., Powell, A.L., 2016. Ripening of tomato fruit and susceptibility to botrytis cinerea. Botrytis–the fungus, the pathogen and its management in agricultural systems, pp. 387–412. https://doi.org/10.1007/978-3-319-23371-0_19.

Brizzolara, S., Minnocci, A., Yembaturova, E., Tonutti, P., 2020. Ultrastructural analysis of berry skin from four grapes varieties at harvest and in relation to postharvest dehydration. Oeno One 54 (4), 1121–1131. https://doi.org/10.20870/oeno-one.2020.54.4.4165.

Bonghi, C., Rizzini, F.M., Gambuti, A., Moio, L., Chkaiban, L., Tonutti, P., 2012. Phenol compound metabolism and gene expression in the skin of wine grape (Vitis vinifera L.) berries subjected to partial postharvest dehydration. Postharvest Biol. Technol. 67, 102–109. https://doi.org/10.1016/j.postharvbio.2012.01.002.

Boss, P.K., Pearce, A.D., Zhao, Y., Nicholson, E.L., Dennis, E.G., Jeffery, D.W., 2015. Potential grape-derived contributions to volatile ester concentrations in wine. Molecules. 20, 7845–7873. https://doi.org/10.3390/molecules20057845.

Buśko, M., Kulik, T., Ostrowska, A., Góral, T., Perkowski, J., 2014. Quantitative volatile compound profiles in fungal cultures of three different Fusarium graminearum chemotypes. FEMS Microbiol. Lett. 359 (1), 85–93. https://doi.org/10.1111/1574-6968.12569.

Coertze, S., Holz, G., 2002. Epidemiology of Botrytis cinerea on grape: wound infection by dry, airborne conidia. South Afr. J. Enol. Viticult. 23 (2), 72–91. https://doi.org/10.21548/23-2-2157.

Costantini, V., Bellincontro, A., De Santis, D., Botondi, R., Mencarelli, F., 2006. Metabolic changes of Malvasia grapes for wine production during postharvest drying. J. Agric. Food Chem. 54 (9), 3334–3340. https://doi.org/10.1021/jf053117l.

Cova, C.M., Rincón, E., Espinosa, E., Serrano, L., Zuliani, A., 2022. Paving the way for a green transition in the design of sensors and biosensors for the detection of volatile organic compounds (VOCs). Biosensors 12 (2), 51. https://doi.org/10.3390/bios12020051.

Dennis, E.G., Keyzers, R.A., Kalua, C.M., Maffei, S.M., Nicholson, E.L., Boss, P.K., 2012. Grape contribution to wine aroma: production of hexyl acetate, octyl acetate, and benzyl acetate during yeast fermentation is dependent upon precursors in the must. J. Agric. Food Chem. 60 (10), 2638–2646. https://doi.org/10.1021/jf2042517.

De Simone, N., Pace, B., Grieco, F., Chimienti, M., Tyibilika, V., Santoro, V., Russo, P., 2020. Botrytis cinerea and table grapes: A review of the main physical, chemical, and

bio-based control treatments in post-harvest. Foods. 9 (9), 1138. https://doi.org/10.3390/foods9091138.
Droby, S., Lichter, A., 2007. Post-harvest Botrytis infection: etiology, development and management. Botrytis: Biology, Pathology and Control. Springer Netherlands, Dordrecht, pp. 349–367. https://doi.org/10.1007/978-1-4020-2626-3_19.
Fall, R., Karl, T., Jordan, A., Lindinger, W., 2001. Biogenic C5 VOCs: release from leaves after freeze–thaw wounding and occurrence in air at a high mountain observatory. Atmos. Environ. 35 (22), 3905–3916. https://doi.org/10.1016/S1352-2310(01)00141-8.
Fisher, A.J., Grimes, H.D., Fall, R., 2003. The biochemical origin of pentenol emissions from wounded leaves. Phytochemistry 62 (2), 159–163. https://doi.org/10.1016/S0031-9422(02)00521-6.
González, C., Brito, N., Sharon, A., 2015. Infection process and fungal virulence factors. Botrytis–the fungus, the pathogen and its management in agricultural systems, pp. 229–246. https://doi.org/10.1007/978-3-319-23371-0_12.
Gorman, Z., Tolley, J.P., Koiwa, H., Kolomiets, M.V., 2021. The synthesis of pentyl leaf volatiles and their role in resistance to anthracnose leaf blight. Front. Plant Sci. 12, 719587. https://doi.org/10.3389/fpls.2021.719587.
Heiden, A.C., Kobel, K., Langebartels, C., Schuh-Thomas, G., Wildt, J., 2003. Emissions of oxygenated volatile organic compounds from plants part I: Emissions from lipoxygenase activity. J. Atmos. Chem. 45, 143–172. https://doi.org/10.1023/A:1024069605420.
He, Y., Borrego, E.J., Gorman, Z., Huang, P.C., Kolomiets, M.V., 2020. Relative contribution of LOX10, green leaf volatiles and JA to wound-induced local and systemic oxylipin and hormone signature in Zea mays (maize). Phytochemistry 174, 112334. https://doi.org/10.1016/j.phytochem.2020.112334.
Holz, G., Gütschow, M., Coertze, S., Calitz, F.J., 2003. Occurrence of Botrytis cinerea and subsequent disease expression at different positions on leaves and bunches of grape. Plant Dis. 87 (4), 351–358. https://doi.org/10.1094/PDIS.2003.87.4.351.
Jansen, R.M.C., Miebach, M., Kleist, E., Van Henten, E.J., Wildt, J., 2009. Release of lipoxygenase products and monoterpenes by tomato plants as an indicator of Botrytis cinerea-induced stress. Plant Biol. 11 (6), 859–868. https://doi.org/10.1111/j.1438-8677.2008.00183.x.
Jansen, R.M.C., Hofstee, J.W., Wildt, J., Vanthoor, B.H.E., Verstappen, F.W.A., Takayama, K., Van Henten, E.J., 2010. Health monitoring of plants by their emitted volatiles: A model to predict the effect of Botrytis cinerea on the concentration of volatiles in a large-scale greenhouse. Biosyst. Eng. 106 (1), 37–47. https://doi.org/10.1016/j.biosystemseng.2010.01.009.
Jardine, K., Barron-Gafford, G.A., Norman, J.P., Abrell, L., Monson, R.K., Meyers, K.T., Huxman, T.E., 2012. Green leaf volatiles and oxygenated metabolite emission bursts from mesquite branches following light–dark transitions. Photosynth. Res. 113, 321–333. https://doi.org/10.1007/s11120-012-9746-5.
Jiang, L., Qiu, Y., Dumlao, M.C., Donald, W.A., Steel, C.C., Schmidtke, L.M., 2023. Detection and prediction of Botrytis cinerea infection levels in wine grapes using volatile analysis. Food Chem. 421, 136120. https://doi.org/10.1016/j.foodchem.2023.136120.
Mencarelli, F., Tonutti, P., 2013. Sweet, Reinforced and Fortified Wines: Grape Biochemistry, Technology and Vinification. John Wiley & Sons.
Modesti, M., Alfieri, G., Chieffo, C., Mencarelli, F., Vannini, A., Catalani, A., Bellincontro, A., 2024. Destructive and non-destructive early detection of postharvest noble rot (Botrytis cinerea) in wine grapes aimed at producing high-quality wines. J. Sci. Food Agric. 104 (4), 2314–2325. https://doi.org/10.1002/jsfa.13120.
Myung, K., 2005. Biosynthesis of Trans-2-Hexenal in Response to Wounding in Strawberry Fruit and Interaction of Trans-2-Hexenal with Botrytis cinerea. University of Kentucky.
Pellegrini, E., Cioni, P.L., Francini, A., Lorenzini, G., Nali, C., Flamini, G., 2012. Volatiles emission patterns in poplar clones varying in response to ozone. J. Chem. Ecol. 38, 924–932. https://doi.org/10.1007/s10886-012-0162-2.
Pertot, I., Giovannini, O., Benanchi, M., Caffi, T., Rossi, V., Mugnai, L., 2017. Combining biocontrol agents with different mechanisms of action in a strategy to control Botrytis cinerea on grapevine. Crop Protect. 97, 85–93. https://doi.org/10.1016/j.cropro.2017.01.010.
Piesik, D., Pańka, D., Delaney, K.J., Skoczek, A., Lamparski, R., Weaver, D.K., 2011. Cereal crop volatile organic compound induction after mechanical injury, beetle herbivory (Oulema spp.), or fungal infection (Fusarium spp.). J. Plant Physiol. 168 (9), 878–886. https://doi.org/10.1016/j.jplph.2010.11.010.
Piombino, P., Genovese, A., Rustioni, L., Moio, L., Failla, O., Bellincontro, A., Mencarelli, F., 2022. Free and glycosylated green leaf volatiles, lipoxygenase and alcohol dehydrogenase in defoliated Nebbiolo grapes during postharvest dehydration. Aust. J. Grape Wine Res. 28 (1), 107–118. https://doi.org/10.1111/ajgw.12521.
Rizzini, F.M., Bonghi, C., Tonutti, P., 2009. Postharvest water loss induces marked changes in transcript profiling in skins of wine grape berries. Postharvest Biol. Technol. 52 (3), 247–253. https://doi.org/10.1016/j.postharvbio.2008.12.004.
Rotolo, C., De Miccolis Angelini, R.M., Dongiovanni, C., Pollastro, S., Fumarola, G., Di Carolo, M., Faretra, F., 2018. Use of biocontrol agents and botanicals in integrated management of Botrytis cinerea in table grape vineyards. Pest. Manage Sci. 74 (3), 715–725. https://doi.org/10.1002/ps.4767.
Sanmartin, C., Modesti, M., Venturi, F., Brizzolara, S., Mencarelli, F., Bellincontro, A., 2021. Postharvest water loss of wine grape: when, what and why. Metabolites. 11 (5), 318. https://doi.org/10.3390/metabo11050318.
Santos, H., Augusto, C., Reis, P., Rego, C., Figueiredo, A.C., Fortes, A.M., 2022. Volatile metabolism of wine grape trincadeira: impact of infection with botrytis cinerea. Plants 11 (1), 141. https://doi.org/10.3390/plants11010141.
Scholthof, K.B.G., Adkins, S., Czosnek, H., Palukaitis, P., Jacquot, E., Hohn, T., Foster, G. D., 2011. Top 10 plant viruses in molecular plant pathology. Mol. Plant Pathol. 12 (9), 938–954. https://doi.org/10.1111/j.1364-3703.2011.00752.x.
Schueuermann, C., Steel, C.C., Blackman, J.W., Clark, A.C., Schwarz, L.J., Moraga, J., Schmidtke, L.M, 2019. A GC–MS untargeted metabolomics approach for the classification of chemical differences in grape juices based on fungal pathogen. Food Chem. 270, 375–384. https://doi.org/10.1016/j.foodchem.2018.07.057.
Scutareanu, P., Drukker, B., Bruin, J., Posthumus, M.A., Sabelis, M.W., 1997. Volatiles from Psylla-infested pear trees and their possible involvement in attraction of anthocorid predators. J. Chem. Ecol. 23, 2241–2260. https://doi.org/10.1023/B:JOEC.0000006671.53045.16.
Wakai, J., Kusama, S., Nakajima, K., Kawai, S., Okumura, Y., Shiojiri, K., 2019. Effects of trans-2-hexenal and cis-3-hexenal on post-harvest strawberry. Sci. Rep. 9 (1), 10112. https://doi.org/10.1038/s41598-019-46307-4.
Wang, S., Takahashi, H., Saito, T., Okawa, K., Ohara, H., Shishido, M., Ikeura, H., Kondo, S., 2015. Jasmonate application influences endogenous abscisic acid, jasmonic acid and aroma volatiles in grapes infected by a pathogen (Glomerella cingulata). Sci. Hortic. 192, 166–172. https://doi.org/10.1016/j.scienta.2015.06.001.
Williamson, B., Tudzynski, B., Tudzynski, P., Van Kan, J.A., 2007. Botrytis cinerea: the cause of grey mould disease. Mol. Plant Pathol. 8 (5), 561–580. https://doi.org/10.1111/j.1364-3703.2007.00417.x.
Zamboni, A., Minoia, L., Ferrarini, A., Tornielli, G.B., Zago, E., Delledonne, M., Pezzotti, M., 2008. Molecular analysis of post-harvest withering in grape by AFLP transcriptional profiling. J. Exp. Bot. 59 (15), 4145–4159. https://doi.org/10.1093/jxb/ern256.
Zenoni, S., Fasoli, M., Guzzo, F., Dal Santo, S., Amato, A., Anesi, A., Tornielli, G.B., 2016. Disclosing the molecular basis of the postharvest life of berry in different grapevine genotypes. Plant Physiol. 172 (3), 1821–1843. https://doi.org/10.1104/pp.16.00865.
Zenoni, S., Amato, A., D'Incà, E., Guzzo, F., Tornielli, G.B., 2020. Rapid dehydration of grape berries dampens the post-ripening transcriptomic program and the metabolite profile evolution. Hortic. Res. 7. https://doi.org/10.1038/s41438-020-00362-5.
Zoccatelli, G., Zenoni, S., Savoi, S., Dal Santo, S., Tononi, P., Zandonà, V., Tornielli, G.B., 2013. Skin pectin metabolism during the postharvest dehydration of berries from three distinct grapevine cultivars. Aust. J. Grape Wine Res. 19 (2), 171–179. https://doi.org/10.1111/ajgw.12014.